

# PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2002-238580

(43)Date of publication of application : 27.08.2002

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(51)IntCl.

C12N 15/09

A01H 5/00

C12N 5/10

C12N 9/10

C12P 21/02

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(21)Application number : 2001-044359

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(22)Date of filing : 20.02.2001

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(54) PLANT CELL HAVING ANIMAL-TYPE SUGAR CHAIN-ADDING FUNCTION

(57)Abstract:

PROBLEM TO BE SOLVED : To provide a plant cell having an animal-type sugar chain.

SOLUTION : A plant cell having an animal-type sugar chain-adding function. The plant cell is prepared by introducing a gene encoding an animal-derived enzyme which can transfer N-acetylglucosamine into a mannose residue of a sugar chain contained in a sugar protein.

(19) 日本国特許庁 (J P)

(12) 公開特許公報 (A)

(11) 特許出願公開番号  
特開2002-238580  
(P2002-238580A)

(43) 公開日 平成14年8月27日(2002.8.27)

(51) Int.Cl. <sup>7</sup>	識別記号	F I	テ-マコ-ト*(参考)
C 1 2 N 15/09	Z N A	A 0 1 H 5/00	A 2 B 0 3 0
A 0 1 H 5/00		C 1 2 N 9/10	4 B 0 2 4
C 1 2 N 5/10		C 1 2 P 21/02	C 4 B 0 5 0
9/10		C 1 2 N 15/00	Z N A A 4 B 0 6 4
C 1 2 P 21/02		5/00	C 4 B 0 6 5
審査請求 未請求 請求項の数6 O L (全 32 頁)			

(21) 出願番号 特願2001-44359(P2001-44359)

(22) 出願日 平成13年2月20日(2001.2.20)

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(54) 【発明の名称】 動物型糖鎖付加機能を持つ植物細胞

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y æ fi æ ^ % L • Ø 麗  
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N., Pierce, M. Isolation, Characterization, and Expression of a cDNA Encoding N-Acetylglucosaminyltransferase V. (1993) J. Biol. Chem. 268, 15381-15388

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% B } P U " A S 變 y ¥ 變

表2. 使用菌株

株	遺伝子型および特徴	参考文献
Escherichia coli JM109	recA1, endA1, gyrA96, thi, hsdR 17, supE 44, relA1, Δ ( lac-proAB ) /F' [traD 36, proAB', lacI <sup>q</sup> , lacZΔM15]	Yanisch-Perron C ら (*)
Agrobacterium tumefaciens LBA4404	Str <sup>r</sup> , Rif <sup>r</sup> バイナリーベクターのT-DNA領域の 移入を容易にするために必要な、 トランスに作用する病原性 (virul ence) 機能を有する	Jen, G.C.ら (** )

(\*) Yanisch-Perron C, Vieira J, Messing J. Improved M13 phage cloning  
g vectors and host strains: nucleotide sequences of the M13mp18 and pUC1  
9 vectors. (1985) Gene 33, 103-119

(\*\*) Jen, G.C., Chilton, M. D. The right border region of pTiT37  
T-DNA is intrinsically more active than the left border region in  
promoting T-DNA transformation. (1986) Proc. Natl. Acad. Sci. USA. 83,  
3895-3899

i P D R D g p v X ~ h 變 y O O F z  
• B y ¥ R

表3. 使用プラスミド

プラスミド	遺伝子型および特徴	参考文献
pBI121		Jfferson, R. A. ら (*)
pRK2013	Km <sup>r</sup> 3親交配により、E. coli から A. tumefaciens にバイナリーベクター中の構築物を移動するために用いた接合プラスミド	Bevan, M. (**)

(\*) Jfferson, R. A., Kavanagh, T. A., Bevan, M. W. GUS fusion:  $\beta$ -glucuronidase as a sensitive and versatile gene fusion in higher plants. (1987) EMBO. J. 6, 3901-3907

(\*\*) Bevan, M. Binary Agrobacterium vectors for plant transformation. (1984) Nucleic Acids. Res. 12, 8711-8721.



Escherichia coli JM109 A w PK2013 ] ~ B  
 Escherichia coli A » 20mg/l J i y O O X T z P U D ^ o R  
 C V 2 Y T | n - R V A P \*  
 E LBA4044 X g 100 mg/l A @ i P U D P D Neasy Plant Mini Kit (Q  
 s 100 mg/l 2 Y T | 28 A Q { IAGEN) p c ~ s ^ o R  
 %B c ~ t % a A » c ~  
 y O O X O z e | { t  
 L A W (10,000 rpm, 1 min) A » ~ ~ y O O X U z i P U D  
 Y T | n - P æ 28 Y T | n  
 % B V c [ u ~ w 10  
 E t A I  
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 L A R ¶ 28 Y T | n a h z  
 Q œ | { % a A P  
 y O O X P z P S D ^  
 d An, G., Ebert, P. R., Mitra,  
 A. and Ha, S. B. (1988) Binary vectors. In plant M 20  
 olecular Biology manual, A3, 1-19, AcademicDordrec  
 ht j ] ` 28  
 y O O X Q z i P S D P D  
 R O O } C [ t 5 ml  
 E A ^ 25 27 • x 20rpm a  
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 | { E ml V | 5  
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 t 100 kg/l l LS t 5ml  
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 y O O X S z P T D ^ o R  
 \*  
 DNeasy Plant Mini Kit ( QIAGEN ) p c B ^ A n 72 4 " B " n A A j |  
 o R | { E t f 50 C } [ `







	27		28
Phe Lys Lys Thr Leu Gly Pro Ser Trp Val His Tyr Gln Cys Met Leu			
360		365	370
cga gtc ctt gat tca ttt ggt act gaa ccc gaa ttt aat cat gca aat			1325
Arg Val Leu Asp Ser Phe Gly Thr Glu Pro Glu Phe Asn His Ala Asn			
375		380	385
tat gcc caa tcg aaa ggc cac aag acc cct tgg gga aaa tgg aat ctg			1373
Tyr Ala Gln Ser Lys Gly His Lys Thr Pro Trp Gly Lys Trp Asn Leu			
	395		400
aac cct cag cag ttt tat acc atg ttc cct cat acc cca gac aac agc			1421
Asn Pro Gln Gln Phe Tyr Thr Met Phe Pro His Thr Pro Asp Asn Ser			
	410		415
ttt ctg ggg ttt gtg gtt gag cag cac ctg aac tcc agt gat atc cac			1469
Phe Leu Gly Phe Val Val Glu Gln His Leu Asn Ser Ser Asp Ile His			
	425		430
cac att aat gaa atc aaa agg cag aac cag tcc ctt gtg tat ggc aaa			1517
His Ile Asn Glu Ile Lys Arg Gln Asn Gln Ser Leu Val Tyr Gly Lys			
	440		445
gtg gat agc ttc tgg aag aat aag aag atc tac ttg gac att att cac			1565
Val Asp Ser Phe Trp Lys Asn Lys Lys Ile Tyr Leu Asp Ile Ile His			
	455		460
aca tac atg gaa gtg cat gca act gtt tat ggc tcc agc aca aag aat			1613
Thr Tyr Met Glu Val His Ala Thr Val Tyr Gly Ser Ser Thr Lys Asn			
	475		480
att ccc agt tac gtg aaa aac cat ggt atc ctc agt gga cgg gac ctg			1661
Ile Pro Ser Tyr Val Lys Asn His Gly Ile Leu Ser Gly Arg Asp Leu			
	490		495
cag ttc ctt ctt cga gaa acc aag ttg ttt gtt gga ctt ggg ttc cct			1709
Gln Phe Leu Leu Arg Glu Thr Lys Leu Phe Val Gly Leu Gly Phe Pro			
	505		510
tac gag ggc cca gct ccc ctg gaa gct atc gca aat gga tgt gct ttt			1757
Tyr Glu Gly Pro Ala Pro Leu Glu Ala Ile Ala Asn Gly Cys Ala Phe			
	520		525
ctg aat ccc aag ttc aac cca ccc aaa agc agc aaa aac aca gac ttt			1805
Leu Asn Pro Lys Phe Asn Pro Pro Lys Ser Ser Lys Asn Thr Asp Phe			
	535		540
ttc att ggc aag cca act ctg aga gag ctg aca tcc cag cat cct tac			1853
Phe Ile Gly Lys Pro Thr Leu Arg Glu Leu Thr Ser Gln His Pro Tyr			
	555		560
gct gaa gtt ttc atc ggg cgg cca cat gtg tgg act gtt gac ctc aac			1901
Ala Glu Val Phe Ile Gly Arg Pro His Val Trp Thr Val Asp Leu Asn			
	570		575
aat cag gag gaa gta gag gat gca gtg aaa gca att tta aat cag aag			1949
Asn Gln Glu Glu Val Glu Asp Ala Val Lys Ala Ile Leu Asn Gln Lys			
	585		590
att gag cca tac atg cca tat gaa ttt acg tgc gag ggg atg cta cag			1997
Ile Glu Pro Tyr Met Pro Tyr Glu Phe Thr Cys Glu Gly Met Leu Gln			
	600		605
aga atc aat gct ttc att gaa aaa cag gac ttc tgc cat ggg caa gtg			2045



29

Arg Ile Asn Ala Phe Ile Glu Lys Gln Asp Phe Cys His Gly Gln Val  
615 620 625 630  
atg tgg cca ccc ctc agc gcc cta cag gtc aag ctt gct gag ccc ggg 2093  
Met Trp Pro Pro Leu Ser Ala Leu Gln Val Lys Leu Ala Glu Pro Gly  
635 640 645  
cag tcc tgc aag cag gtg tgc cag gag agc cag ctc atc tgc gag cct 2141  
Gln Ser Cys Lys Gln Val Cys Gln Glu Ser Gln Leu Ile Cys Glu Pro  
650 655 660  
tct ttc ttc cag cac ctc aac aag gac aag gac atg ctg aag tac aag 2189  
Ser Phe Phe Gln His Leu Asn Lys Asp Lys Asp Met Leu Lys Tyr Lys  
665 670 675  
gtg acc tgc caa agc tca gag ctg gcc aag gac atc ctg gtg ccc tcc 2237  
Val Thr Cys Gln Ser Ser Glu Leu Ala Lys Asp Ile Leu Val Pro Ser  
680 685 690  
ttt gac cct aag aat aag cac tgt gtg ttt caa ggt gac ctc ctg ctc 2285  
Phe Asp Pro Lys Asn Lys His Cys Val Phe Gln Gly Asp Leu Leu Leu  
695 700 705 710  
ttc agc tgt gca ggc gcc cac ccc agg cac cag agg gtc tgc ccc tgc 2333  
Phe Ser Cys Ala Gly Ala His Pro Arg His Gln Arg Val Cys Pro Cys  
715 720 725  
cgg gac ttc atc aag ggc cag gtg gct ctc tgc aaa gac tgc cta 2378  
Arg Asp Phe Ile Lys Gly Gln Val Ala Leu Cys Lys Asp Cys Leu  
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<212> PRT  
<213> human  
<400> 2  
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20 25 30  
Thr Ile Gln Gln Arg Thr Gln Pro Glu Ser Ser Ser Met Leu Arg Glu  
35 40 45  
Gln Ile Leu Asp Leu Ser Lys Arg Tyr Ile Lys Ala Leu Ala Glu Glu  
50 55 60  
Asn Arg Asn Val Val Asp Gly Pro Tyr Ala Gly Val Met Thr Ala Tyr  
65 70 75 80  
Asp Leu Lys Lys Thr Leu Ala Val Leu Leu Asp Asn Ile Leu Gln Arg  
85 90 95  
Ile Gly Lys Leu Glu Ser Lys Val Asp Asn Leu Val Val Asn Gly Thr  
100 105 110  
Gly Thr Asn Ser Thr Asn Ser Thr Thr Ala Val Pro Ser Leu Val Ala  
115 120 125  
Leu Glu Lys Ile Asn Val Ala Asp Ile Ile Asn Gly Ala Gln Glu Lys  
130 135 140  
Cys Val Leu Pro Pro Met Asp Gly Tyr Pro His Cys Glu Gly Lys Ile  
145 150 155 160  
Lys Trp Met Lys Asp Met Trp Arg Ser Asp Pro Cys Tyr Ala Asp Tyr

31

165 170 175

Gly Val Asp Gly Ser Thr Cys Ser Phe Phe Ile Tyr Leu Ser Glu Val  
180 185 190

Glu Asn Trp Cys Pro His Leu Pro Trp Arg Ala Lys Asn Pro Tyr Glu  
195 200 205

Glu Ala Asp His Asn Ser Leu Ala Glu Ile Arg Thr Asp Phe Asn Ile  
210 215 220

Leu Tyr Ser Met Met Lys Lys His Glu Glu Phe Arg Trp Met Arg Leu  
225 230 235 240

Arg Ile Arg Arg Met Ala Asp Ala Trp Ile Gln Ala Ile Lys Ser Leu  
245 250 255

Ala Glu Lys Gln Asn Leu Glu Lys Arg Lys Arg Lys Lys Val Leu Val  
260 265 270

His Leu Gly Leu Leu Thr Lys Glu Ser Gly Phe Lys Ile Ala Glu Thr  
275 280 285

Ala Phe Ser Gly Gly Pro Leu Gly Glu Leu Val Gln Trp Ser Asp Leu  
290 295 300

Ile Thr Ser Leu Tyr Leu Leu Gly His Asp Ile Arg Ile Ser Ala Ser  
305 310 315 320

Leu Ala Glu Leu Lys Glu Ile Met Lys Lys Val Val Gly Asn Arg Ser  
325 330 335

Gly Cys Pro Thr Val Gly Asp Arg Ile Val Glu Leu Ile Tyr Ile Asp  
340 345 350

Ile Val Gly Leu Ala Gln Phe Lys Lys Thr Leu Gly Pro Ser Trp Val  
355 360 365

His Tyr Gln Cys Met Leu Arg Val Leu Asp Ser Phe Gly Thr Glu Pro  
370 375 380

Glu Phe Asn His Ala Asn Tyr Ala Gln Ser Lys Gly His Lys Thr Pro  
385 390 395 400

Trp Gly Lys Trp Asn Leu Asn Pro Gln Gln Phe Tyr Thr Met Phe Pro  
405 410 415

His Thr Pro Asp Asn Ser Phe Leu Gly Phe Val Val Glu Gln His Leu  
420 425 430

Asn Ser Ser Asp Ile His His Ile Asn Glu Ile Lys Arg Gln Asn Gln  
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Ser Leu Val Tyr Gly Lys Val Asp Ser Phe Trp Lys Asn Lys Lys Ile  
450 455 460

Tyr Leu Asp Ile Ile His Thr Tyr Met Glu Val His Ala Thr Val Tyr  
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Gly Ser Ser Thr Lys Asn Ile Pro Ser Tyr Val Lys Asn His Gly Ile  
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Leu Ser Gly Arg Asp Leu Gln Phe Leu Leu Arg Glu Thr Lys Leu Phe  
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Val Gly Leu Gly Phe Pro Tyr Glu Gly Pro Ala Pro Leu Glu Ala Ile  
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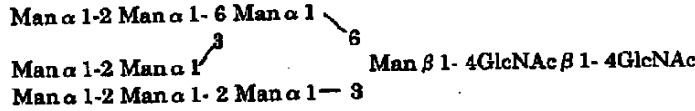
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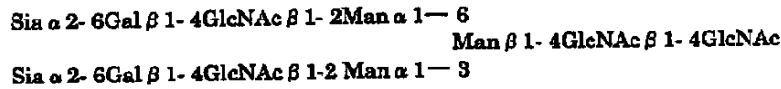
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N-linked chain

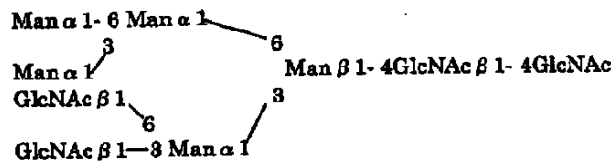
1) High mannose type



2) Complex type

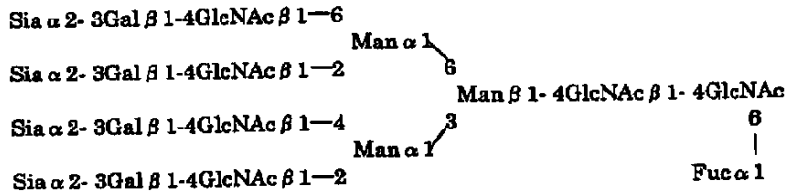


3) Hybrid type

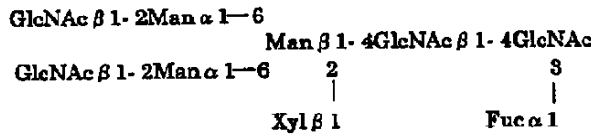


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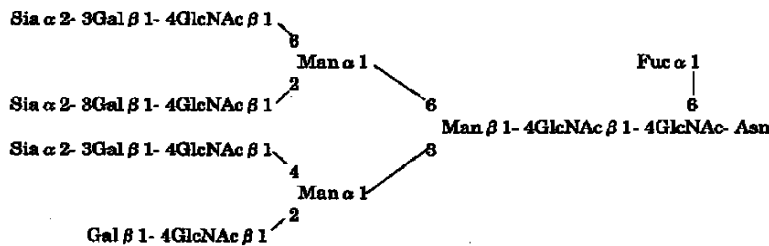
1) Mammalian cell type



2) Plant cell Type



y } E



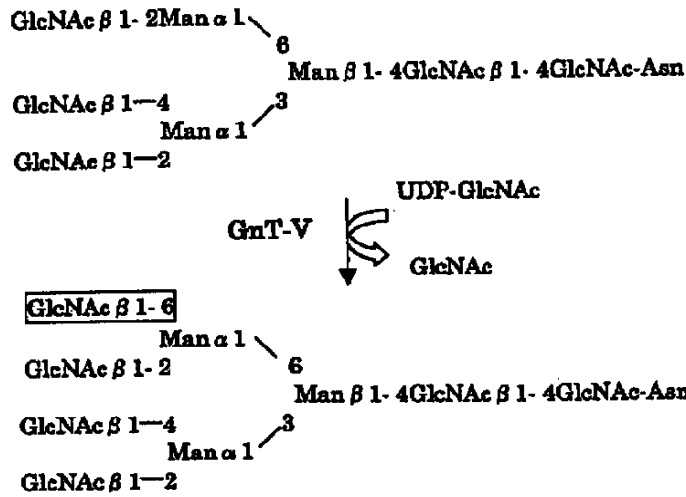
← 先端糖鎖部分 → ← 分岐糖鎖部分 → ← コア糖鎖部分 →  
 【外界との相互作用】 【糖鎖のカバーする空間の増大】 【ペプチド部分の相互作用】

血中クリアランスのシグナル

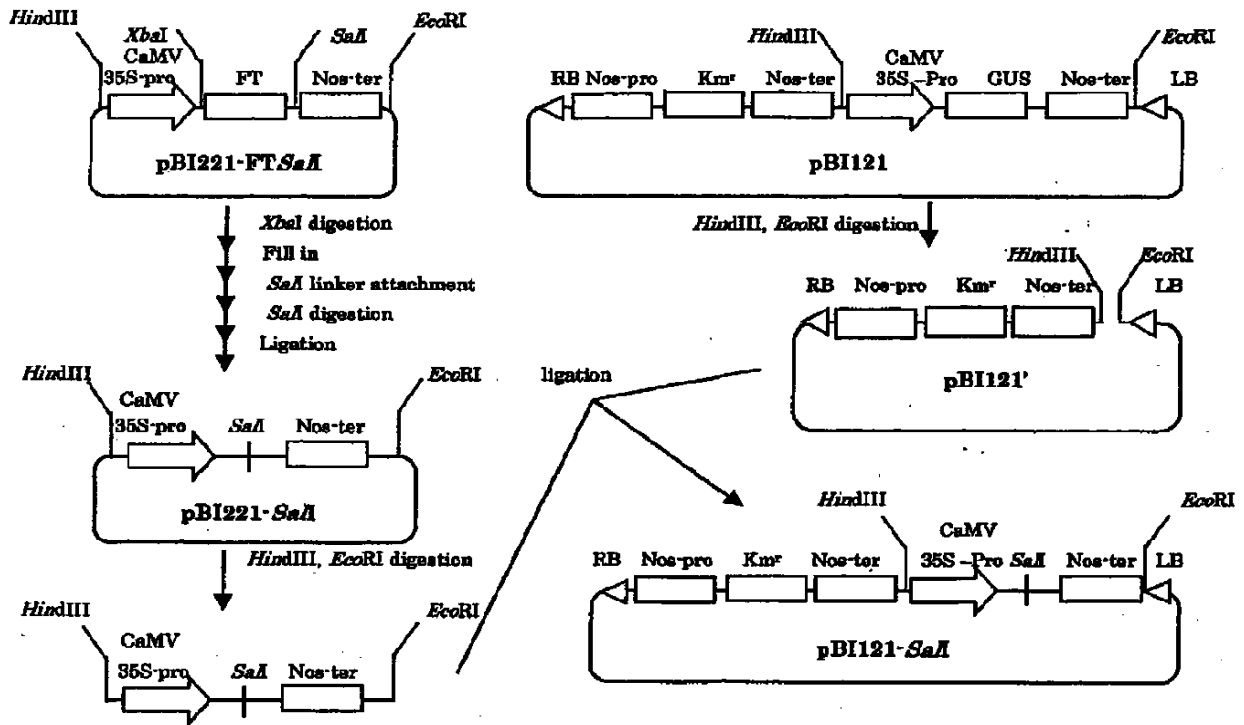
リガンドの立体配置、ホーミング

活性の保持

y } s



y } v



y } z

ATGGCTCTCTTCACTCCGTTGGAAGTTGTCCTCTCAGAAGCTGGGCTTTTTCTGGTGACT  
 METAlaLeuPheThrProTrpLysLeuSerSerGlnLysLeuGlyPhePheLeuValThr  
 TTTGGCTTCATTTGGGGTATGATGCTTCTGCACTTTIACCATCCAGCAGCGAACTCAGCCT  
 PheGlyPheIleTrpGlyMETMETLeuLeuHisPheThrIleGlnGlnArgThrGlnPro  
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 GluSerSerSerMETLeuArgGluGlnIleLeuAspLeuSerLysArgTyrIleLysAla  
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 GCTCAGAAAATGTGTATTGCCTCCTATGGACGGCTACCCCTCACTGTGAGGCAAGATC  
 AlaGlnGluLysCysValLeuProPromETAAspGlyTyrProHisCysGluGlyLysIle  
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 LysTrpMETLysAspMETTrpArgSerAspProCysTyrAlaAspTyrGlyValAspGly  
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 SerThrCysSerPhePheIleTyrLeuSerGluValGluAsnTrpCysProHisLeuPro  
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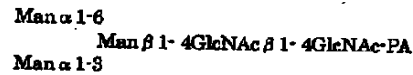
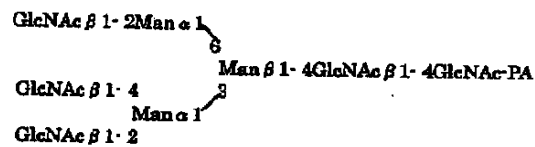
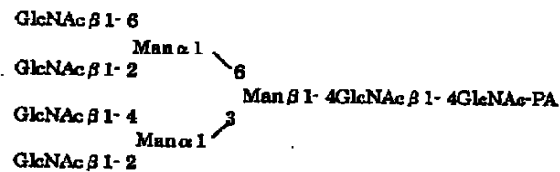
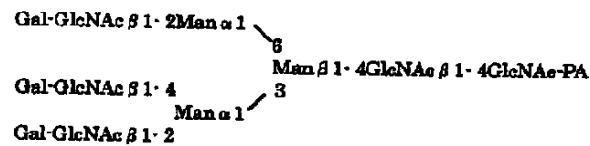
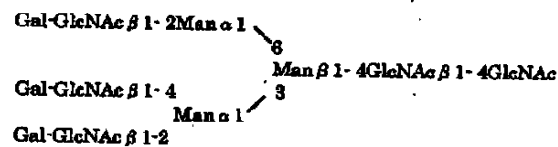
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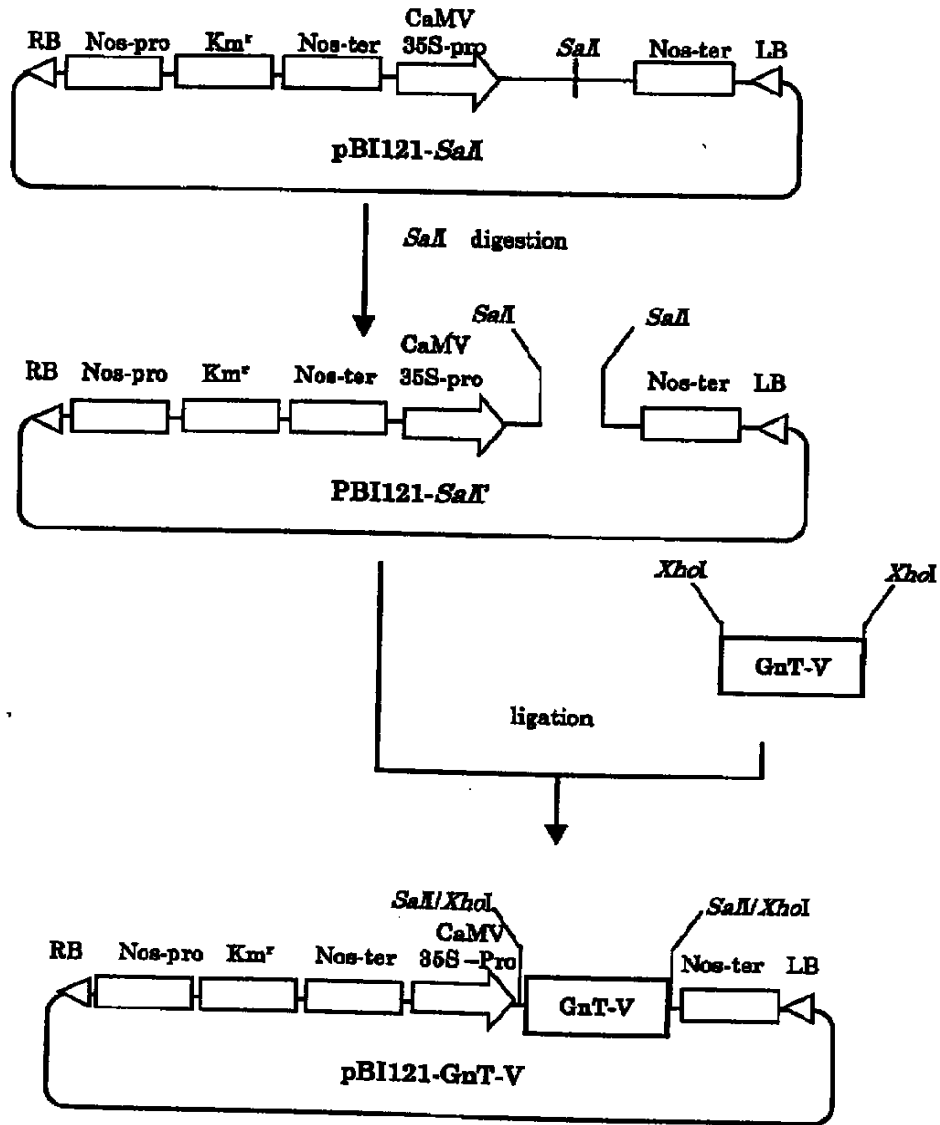
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**PA057****PA016****PA013****PA014****PA002****Sugar chain Gal3Gn8M3**

PA : Pridylamino(group)  
 Gal : Galactose  
 GlcNAc : N-Acetylglucosamine  
 Man : Mannose

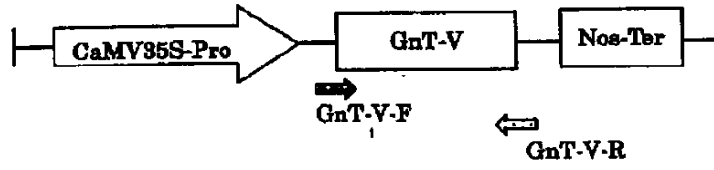


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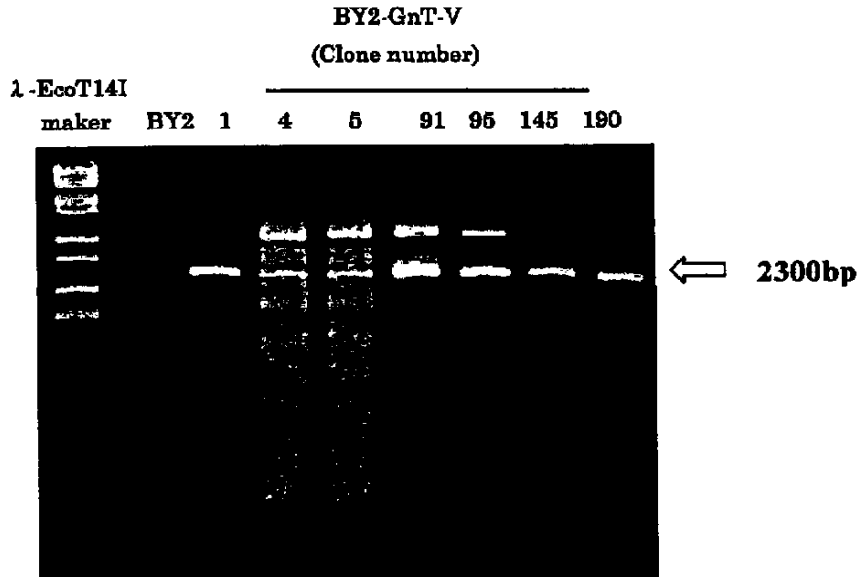


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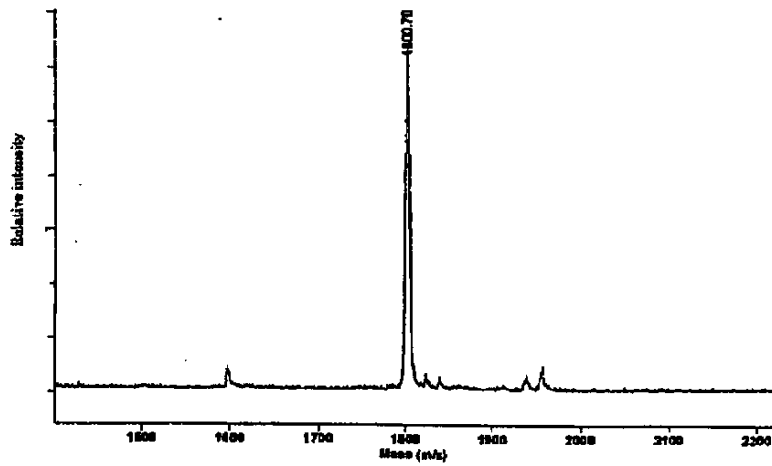
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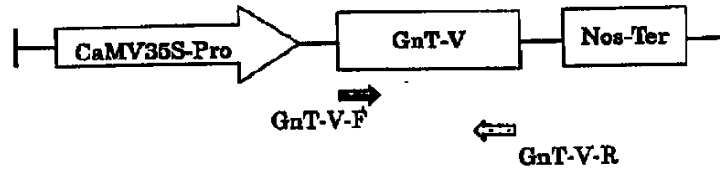


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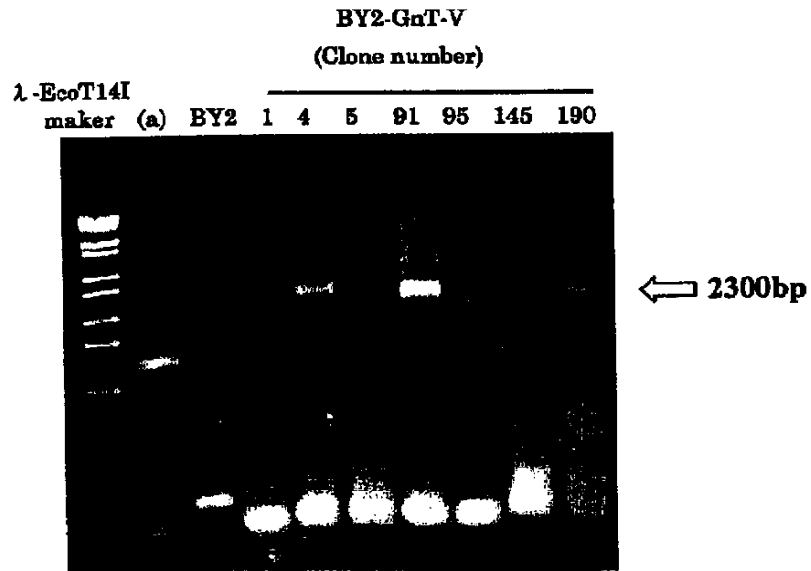


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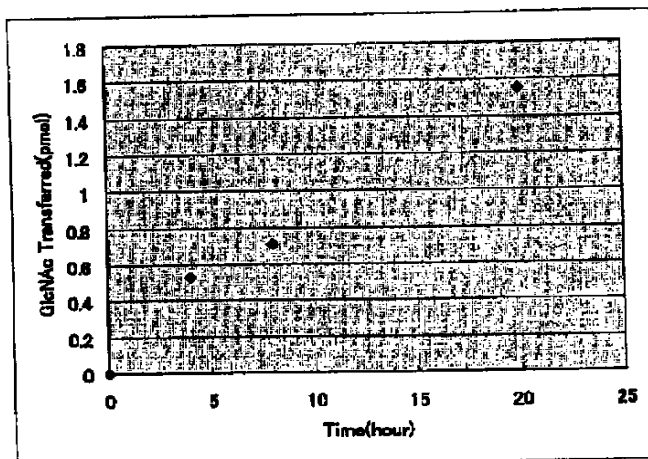
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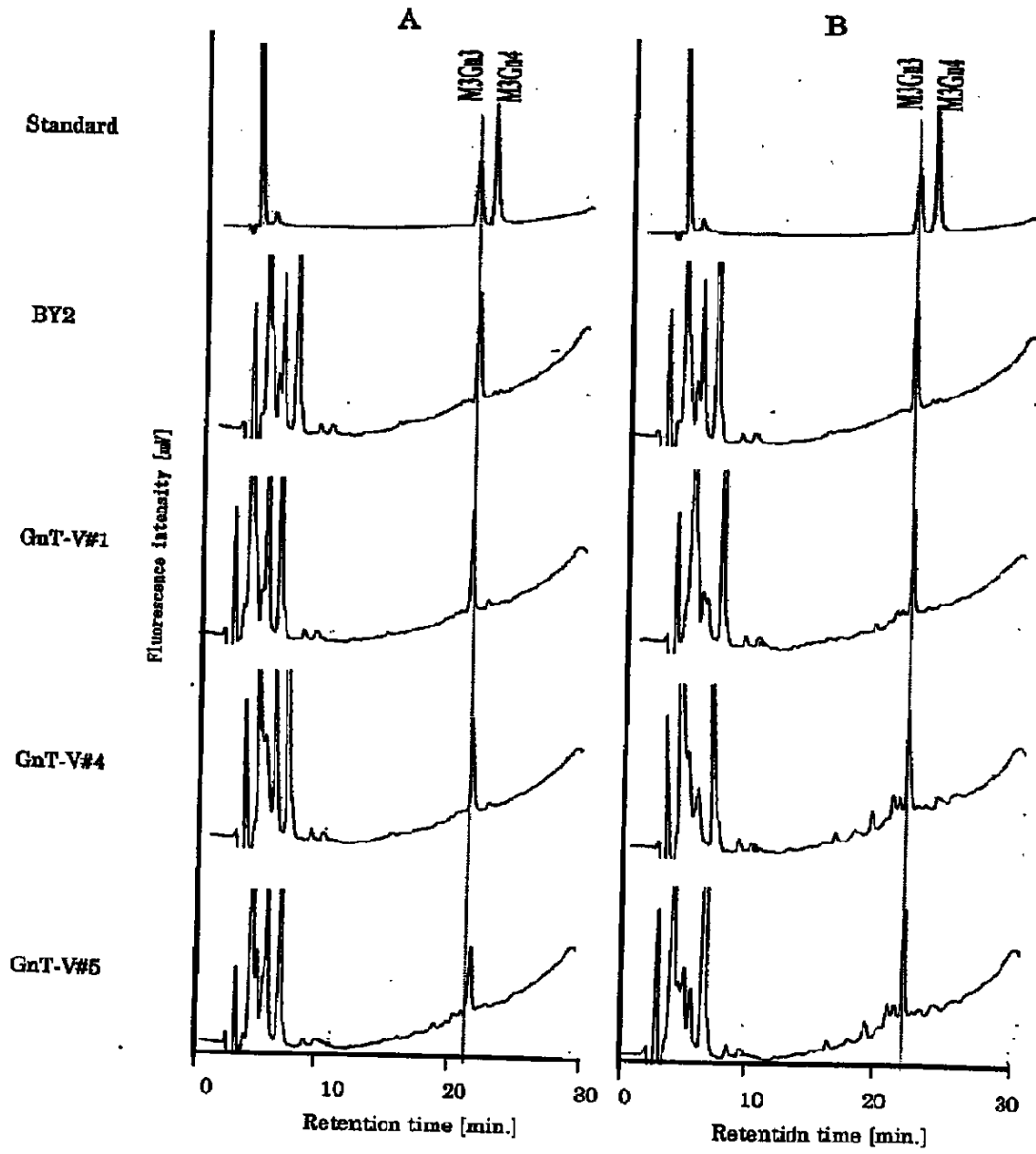


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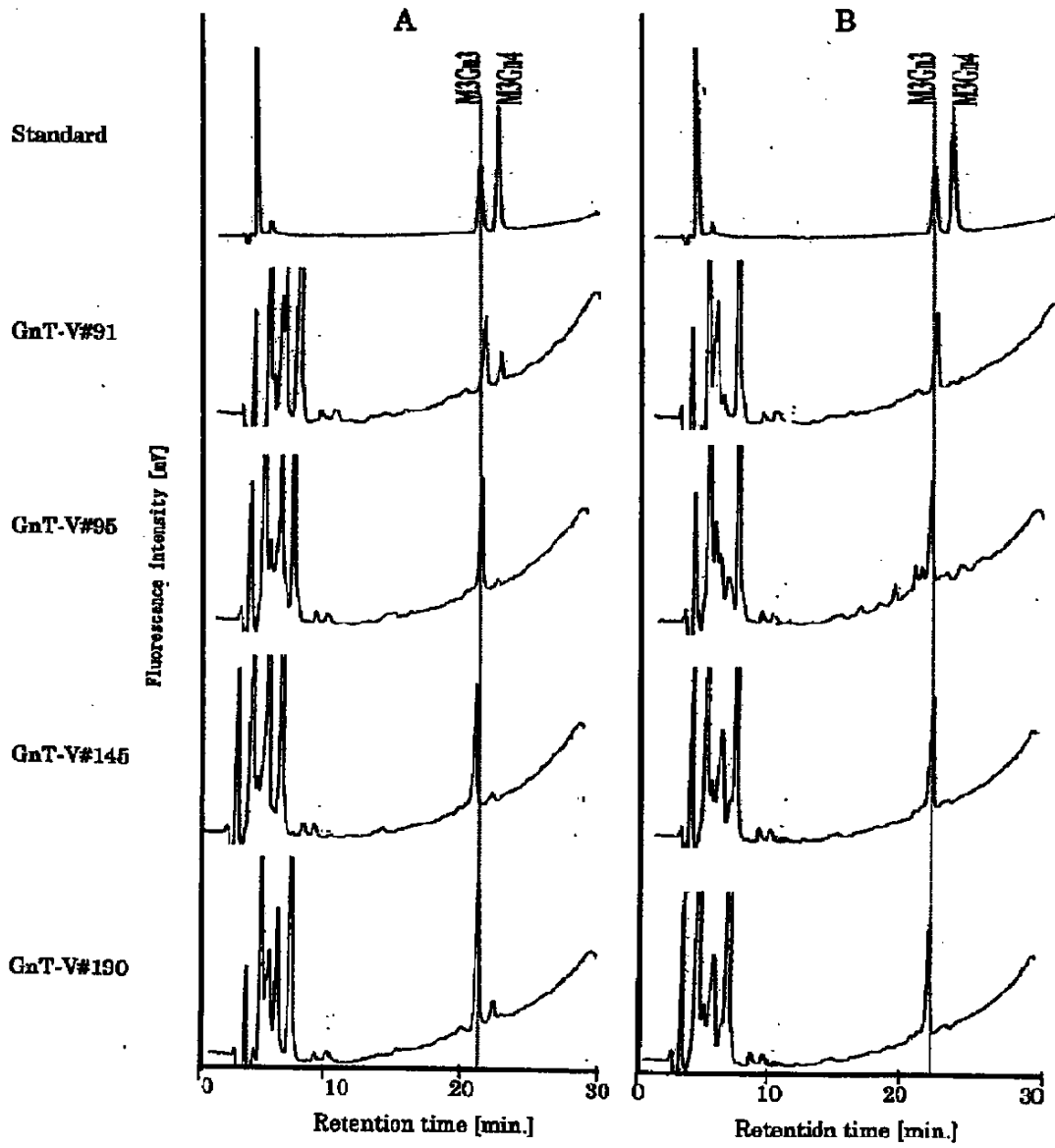




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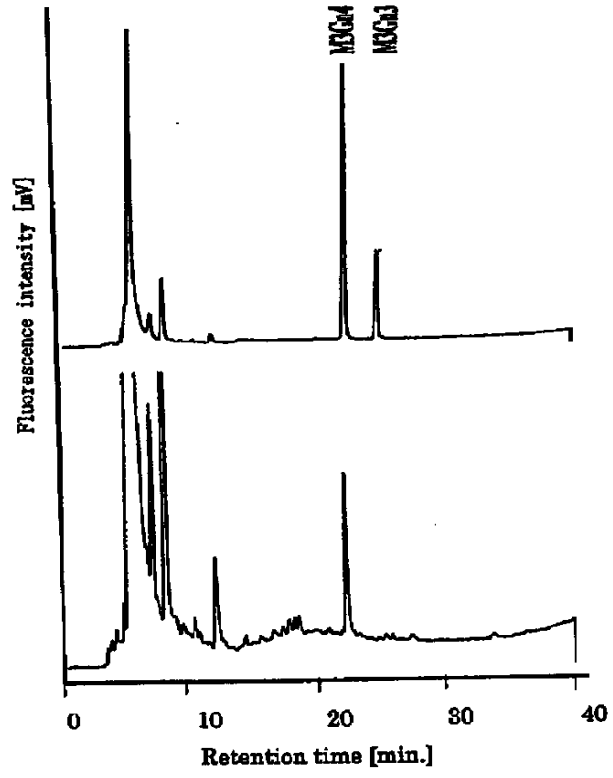
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Standard

GnT-V#91  
Product



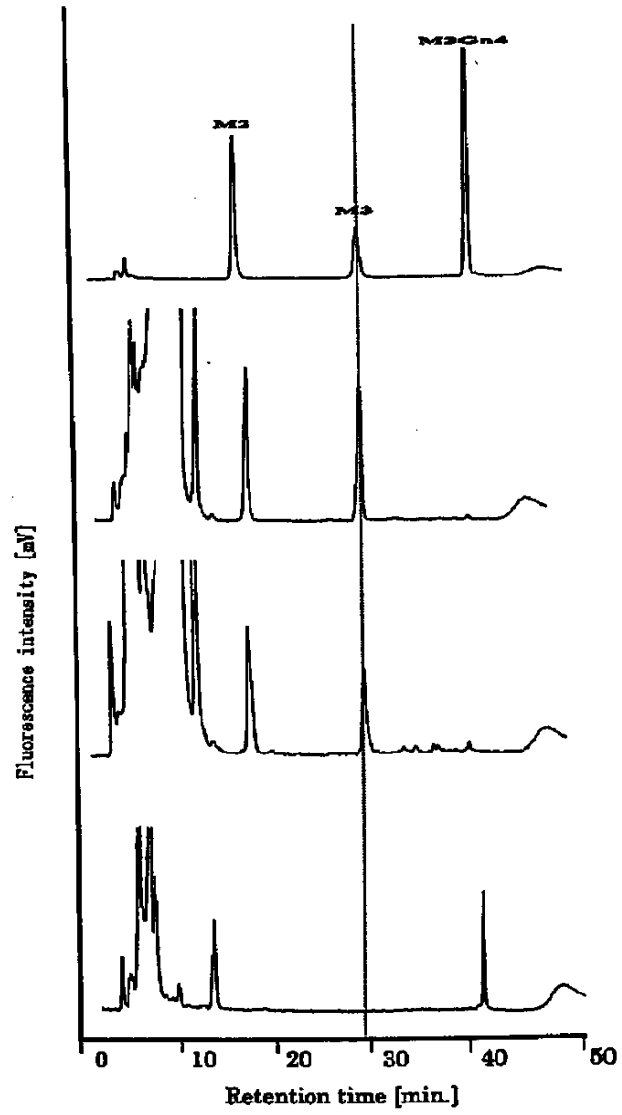
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Standard

PA014 N-ア  
セチルヘキソ  
サミニダーゼ  
処理

BY2-GnT-V#91  
Product N-アセチル  
ヘキソサミニダーゼ  
処理

BY2-GnT-V#91  
Product 酵素未添  
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